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EFFECT OF INFRARED LASER RADIATION ON BIOLOGICAL SYSTEMS

FINAL REPORT

by

GEROGE W. PRATT

LEVEL II

JUNE 1973

Supported by

U.S. Army Medical Research and Development Command
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A low power CO_2 laser has been used to kill a spore preparation of Bacillus Subtilis. The CO_2 laser has been found to be very effective in deactivating these spores. A 65 watt/cm^2 beam reduced the dry live spore population by 3 orders of magnitude in 0.4 seconds as compared to a reduction by one order of magnitude after 60 seconds exposure (150 times as long) to pressurized steam at 130°C. A possible explanation of the high efficiency of laser sterilization may be deactivation by resonant absorption of infrared energy at 10.6 μ. Experiments discussing selected damage in proteins are presented.

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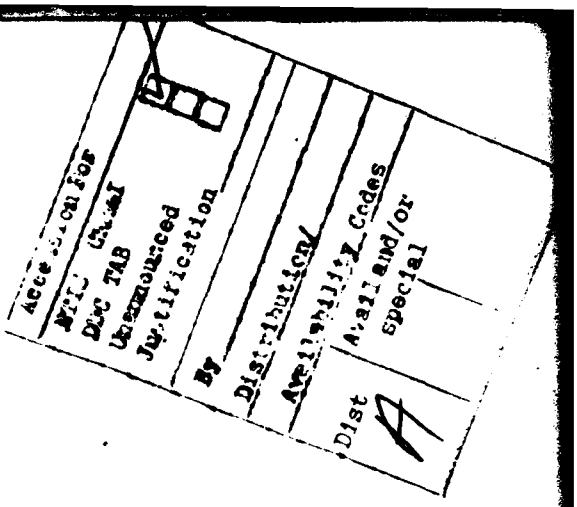
INTRODUCTION

This is the "Final Scientific Report" under Contract No. DADA17-72-C-2101. The title of the contract was "Effect of Infrared Laser Radiation on Biological Systems."

EXPERIMENTAL RESULTS

The first experiments were carried out using a very heat resistant dry spore preparation of Bacillus Subtilis. These spores were initially spread on Al substrates because metals, such as Al, reflect virtually all of the incident CO_2 laser radiation up to very high incident powers. These spores have a decimal reduction time D (that time required to reduce the number of viable organism to $1/10^{th}$ of the original value) of approximately 60 seconds in pressurized steam at 130°C. In a 65 watt/cm^2 laser beam the spore population was reduced by 3 orders of magnitude in 0.4 sec for dry spores and by 5 orders of magnitude for spores with an active water coefficient of 0.8. This is shown in Fig. 1.

The spores are inactivated so rapidly in the CO_2 beam that it is not necessary to disperse them on a metal substrate. A 20 watt/cm^2 beam pulse for $1/20^{th}$ of a second delivers one joule to the target. If all of this energy were absorbed by a paper substrate 10^{-2} cm thick and 1 cm^2 area, the temperature would rise only by 50°C which is well below the rise necessary for ignition. The same energy delivered to a 10 μ thick spore layer would raise the temperature of these spores by roughly 500°C. Assuming approximately equal densities and specific heats the temperature rise of a surface layer δT_L of thickness t_1 on a substrate of thickness t_s is in a very rough approximation



given by

$$\delta T_L = \frac{t_s}{t_l} \delta T_s$$

where T_s is the temperarue rise of the substrate. Experiments carried out where the spore layer was spread over celluiose acetate paper showed that $1/25^{th}$ of a second pulses at the 20 watt operating level completely destroyed the spore activity, but made no visible change in the paper substrate. This result is of considerable practical importance. It means that surface contamination can be rendered sterile on materials that are quite sensitive to heat and which cannot be sterilized using ordinary techniques eg. steam. The list of such materials is extensive including sutures, plastic and rubber catheters, dressings, etc. In addition many materials are unsuitable for radiosterilization with γ rays. Glass darkness and some rubber and plastics deteriorate mechanically. Metallic instruments, vessels, etc. reflect most of the infrared energy until incident power levels in the $K\omega/cm^2$ range are attained. Organisms on the metal surface would, however, absorb the laser energy and be destroyed. The CO_2 and CO lasers offer the very attractive feature of sterilization of metallic surfaces in seconds rather than customary times of several minutes. Flash sterilization of foodstuffs is a further application again where surface contamination is involved.

Similar experiments have been carried out using Streptococcus faecium. The deactivation obtained in 60 seconds in the CO_2 beam was the equivalent to that produced by a radiation dose of 2.5 M rad. This requires 500

minutes from the MIT γ-ray source.

An interesting question is what is the nature of the "heating" process in a laser pulse of the order of 10^{-2} seconds duration if all the energy absorbed were taken up by specific vibrational modes of the irradiated molecules? Certainly 10^{-2} sec is enormously long compared to vibrational periods and to probable molecular relaxation times. One can estimate the molecular relaxation times from the infrared line widths. A line width of 50 cm^{-1} in a 1000 cm^{-1} line corresponds to a relaxation time of 1.2×10^{-13} sec. Therefore, energy absorbed in a given molecular vibrational mode is rapidly dispersed through the excitation spectrum. A 20 watt beam pulsed for $1/20^{th}$ of a second delivers 5×10^{19} photons to a target area of 1 cm^2. This is approximately 10^5 quanta per absorbing bond per second. The deviation from the average number of quanta of vibrational excitation is in a specific absorbing mode is roughly

$$\frac{n - n_o}{\tau} = 10^5 .$$

With $\tau \sim 1.2 \times 10^{-13}$ sec we see that $n - n_o$ is essentially zero. At 10.6 μ each photon carries 0.117 ev and 20 of these photons would be required to break a normal covalent bond. However, a typical hydrogen bond has a binding energy of 0.1 ev. Consequently at these low radiation levels from the CO_2 laser it is almost certain that molecular alterations will be due to disruption of hydrogen bonds effecting total or partial denaturation.

The estimate above pictures individual molecules with an absorbing mode treated as a damped oscillator whose line width is described by a relaxation time τ, which is typically 10^{-12} sec long. Temperature is not well defined at times as short as this. In order to invoke reaction kinetics and heat flow equations enough time must elapse for the transfer of energy between molecules. This is the thermal relaxation time τ_h and is related to the thermal conductivity. Typical values are 10^{-6} - 10^{-7} seconds. Hayes and Wolbarsht[1] point out that laser pulses short compared to τ_h heat up the target before the energy can be conducted away as heat. The temperature rise in

$$\Delta T = FE_{abs}/C$$

where C is the heat capacity of the target particle, E_{abs} the absorbed energy, and F the fraction of the absorbed energy available for heating. F is given by Hayes and Wolbarsht as

$$F = \frac{\tau_h}{\tau_e}\left(1 - e^{-\tau_e/\tau_h}\right)$$

where τ_e is the exposure time. In order to convey some feeling for the numbers involved in heat flow we quote a result of Vassiliadis[2] that a melanin granule 1 μ in diameter with an initial temperature rise ΔT cools to 1/e of ΔT (after the radiation is shut off) in 4×10^{-7} seconds.

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[1] J.R. Hayes and M.L. Wolbarsht, "Laser Applications in Medicine and Biology", Vol 1, Chapter IX, Plenun Press, ed., by M.L. Wolbarsht, New York, (1971).

[2] A. Vassiliadis, Loc. Cit. Chapter VI.

The extent of bond breaking due to heating is described by reaction kinetics. The equilibrium constant K_{eq} for a given bond is

$$K_{eq} = \frac{[\text{number of intact bonds}]}{[\text{number of broken bonds}]}$$

and is related to the bond strength ΔG by

$$K_{eq} = e^{-\Delta G/RT} .$$

A ΔG typical of a hydrogen bond is -2.726 K cal/mole and it leads to the following ratio as a function of temperature:

T°C	K_{eq}
500	5.6
300	9.8
100	30
25	100 .

A ΔG of -27.26 K cal/mole is representative of a covalent bond strength and gives a K_{eq} of $10^{7.5}$ at 500°C. Thus heating a biomaterial to only 300°C breaks 10% of the hydrogen bonds but only one covalent bond in 30 million even at 500°C.

It has been stressed above that the infrared laser allows one to deliver large amounts of energy at a specific frequency and over a time domain ranging from cw to pulses of 10^{-12} sec duration. A key question is

whether or not this energy can indeed be directed into specific bonds in a protein or nucleic acid. If this is successful it will then be a question of whether controlled molecular changes can be brought about. When the exposure time is long compared to intra-molecular relaxation times, then a given vibrational mode will leak away its excitation energy. The heat energy may be somewhat confined in the molecular structure but will more probably feed energy into all of the molecular vibrational and rotational modes. Therefore, the exposure time would seem to be confined to periods of less than 10^{-9} seconds if only one particular bond is to be affected. An exposure time between the intra and inter molecular relaxation time will heat the exposed material so rapidly that the heat cannot escape. This will lead to the maximum temperature rise for a given energy input. One concludes then that sterilization will be most effectively accomplished under these conditions.

A step towards the development of pulse techniques has been taken by a Senior Thesis student, Marshall Schorin.[3] Simple proteins, polyglycine and phenylalanylglycine were pressed in KBr pellets. The infrared transmission spectrum was run on the pellet which was then exposed to pulsed CO_2 radiation and the transmission spectrum run again. Exposure times were long eg. 10^{-1} sec. Therefore, the laser had to act essentially as a heater. Changes in the transmission spectrum of these materials was

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[3] M. Schorin, Senior Thesis Dept. of Electrical Engineering, MIT (1971).

observed and it differed for different parts of the spectrum. No identification has been made of which particular vibrational modes were most affected. Shorin's results are shown in Figures 2, 3, and 4. The main significance of Shorin's results is that he has established a useful technique that can be applied to higher power, shorter duration pulses.

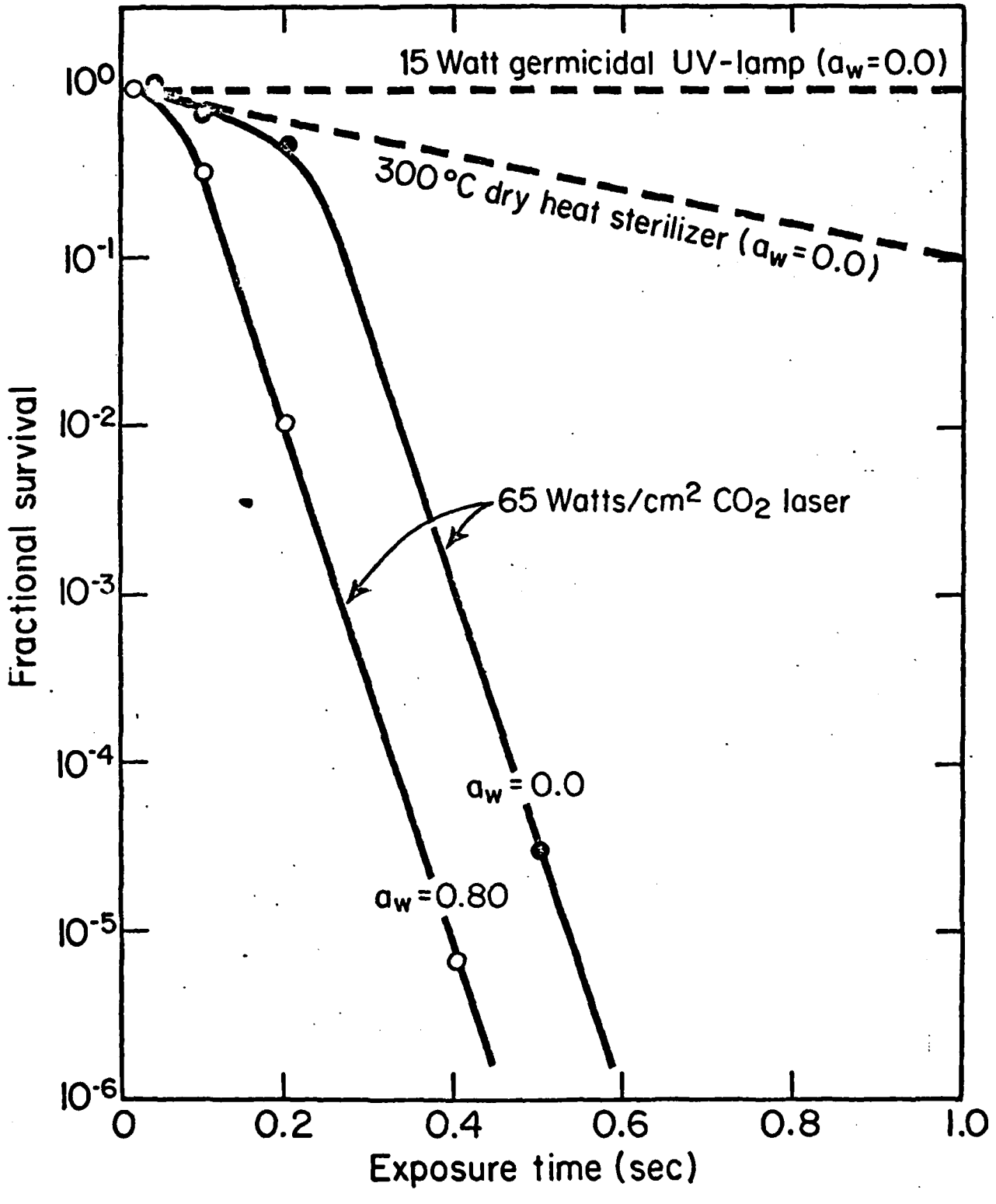
15 Watt germicidal UV-lamp (a_w=0.0)
300 °C dry heat sterilizer (a_w=0.0)
65 Watts/cm^2 CO_2 laser
a_w = 0.0
a_w = 0.80
Fractional survival
10^0
10^{-1}
10^{-2}
10^{-3}
10^{-4}
10^{-5}
10^{-6}
0
0.2
0.4
0.6
0.8
1.0
Exposure time (sec)

Fig. 1.

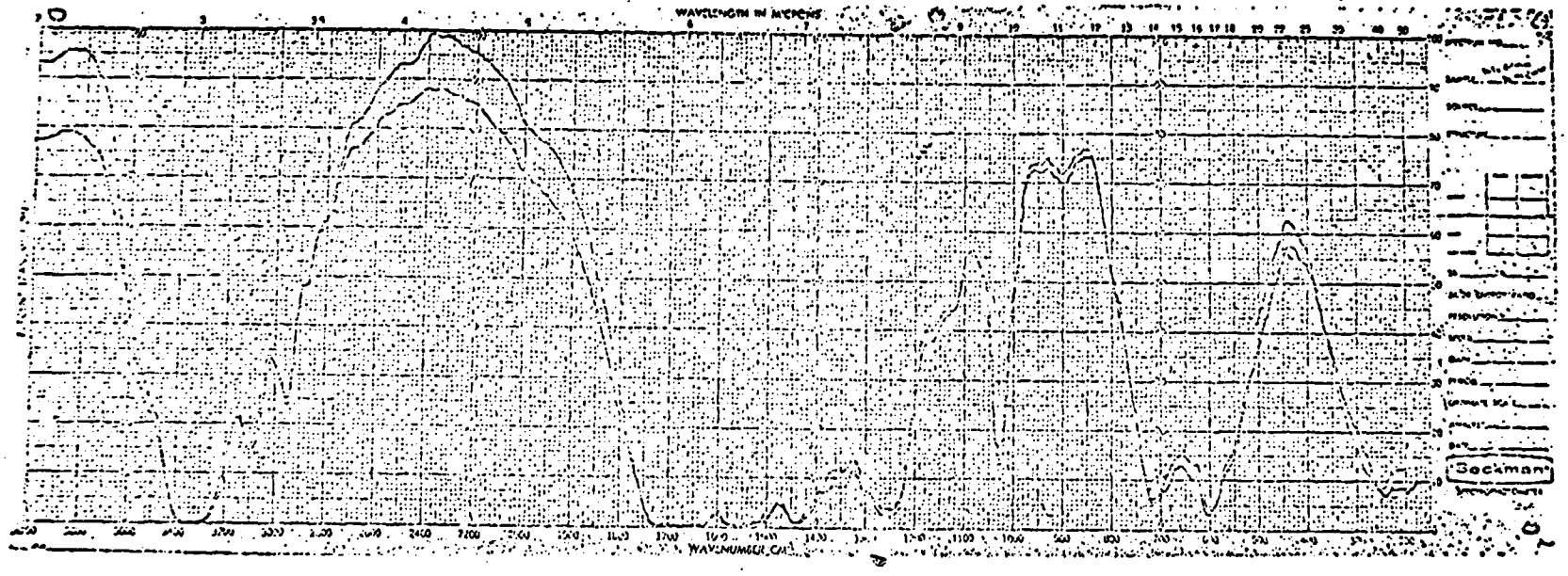

Fig. 2 This graph shows the difference between polyglycine unexposed (broken line) and exposed for a short time only (0.2 sec. @ 60 W.) (solid line).

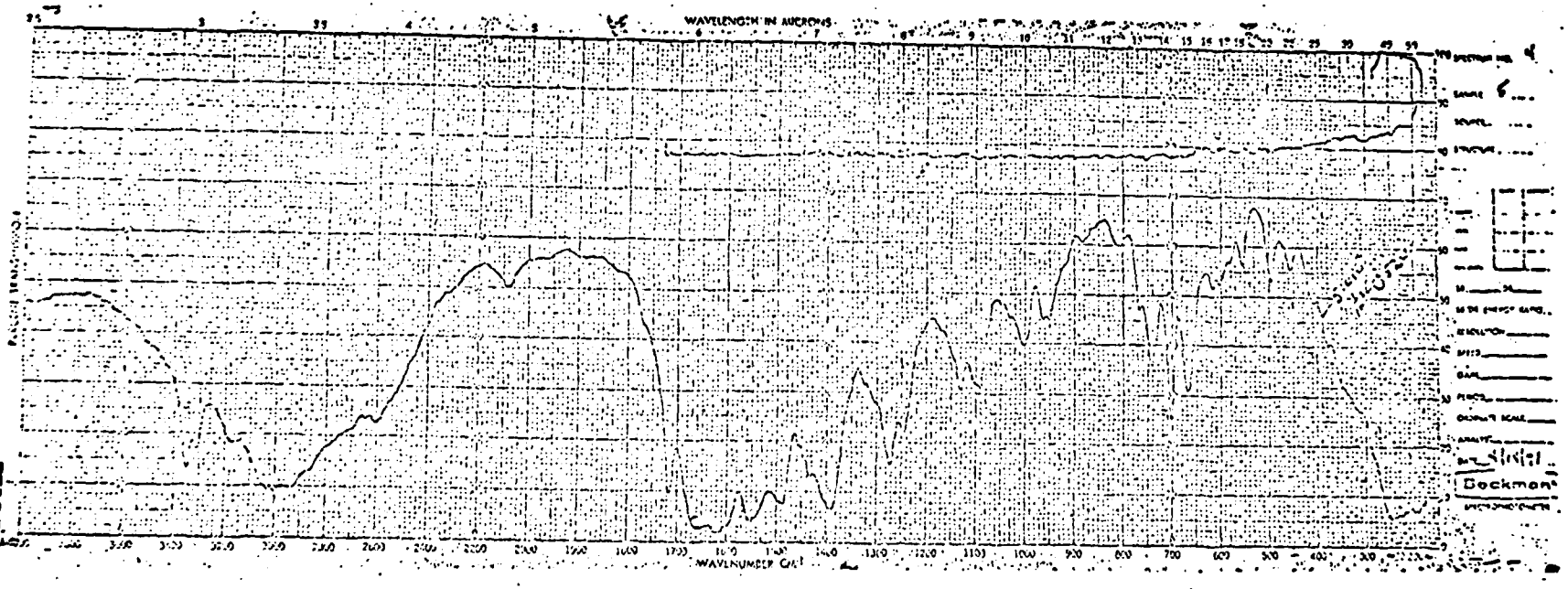

Fig. 3 Phenylalanylglycine after small exposure (0.1 sec. @ 52 W.). (The top (incomplete) trace was used to check the level of the spectrophotometer; ignore it.)

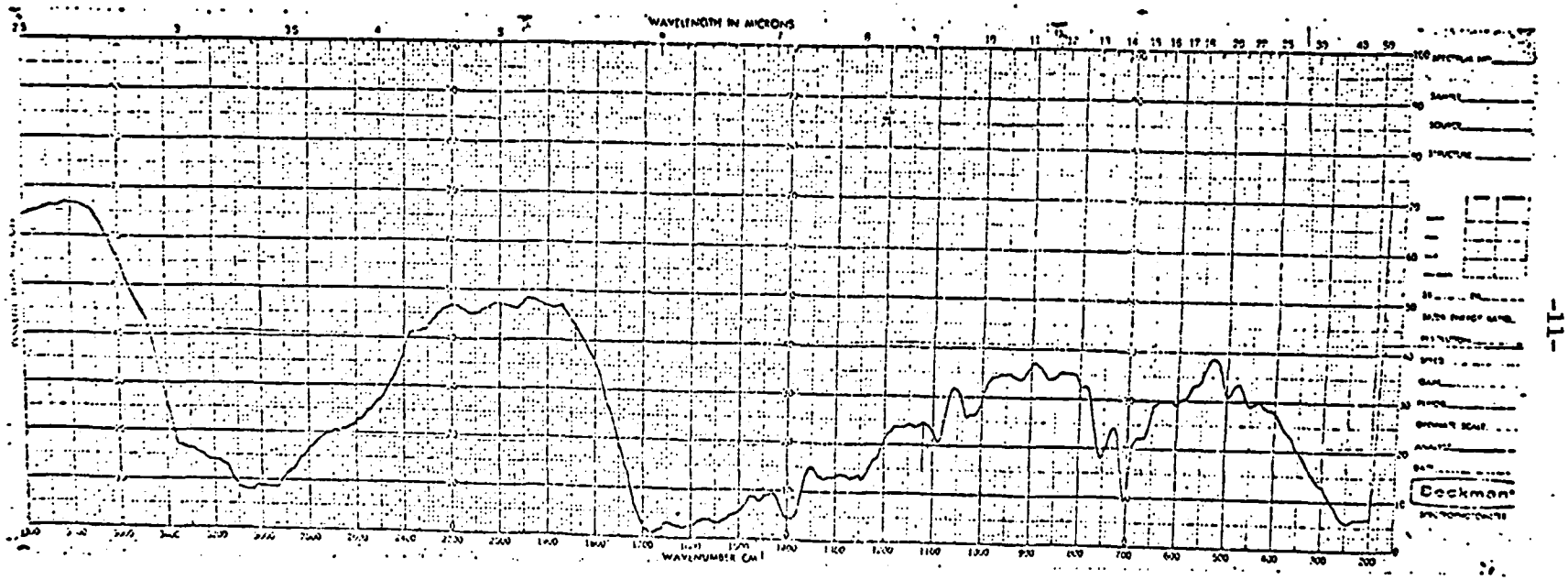

Fig. 4 . This graph was the first one to indicate a change in spectrum after irradiation. It is phenylalanylglycine exposed for 0.5 sec. @ 60 W.

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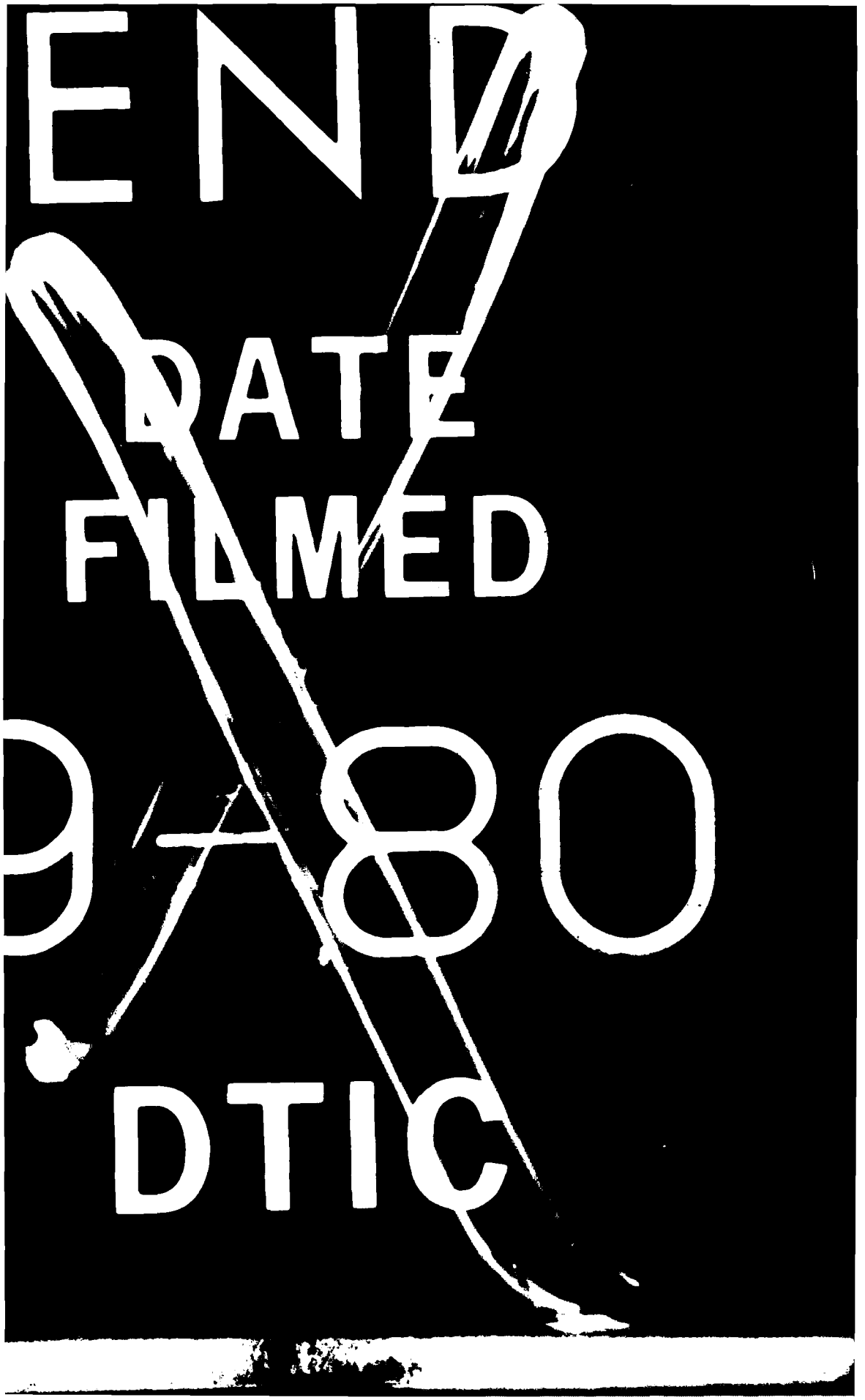
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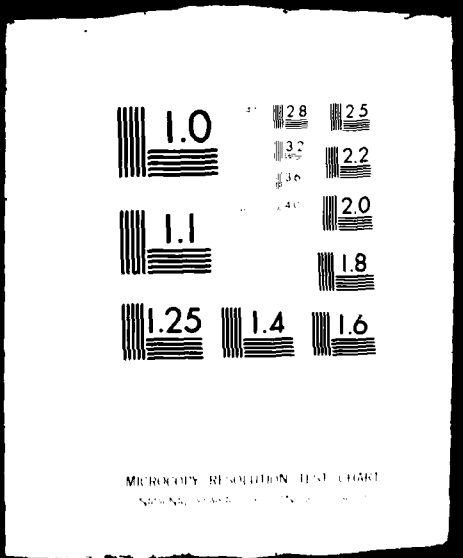

SUPPLEMENTARY

INFORMATION

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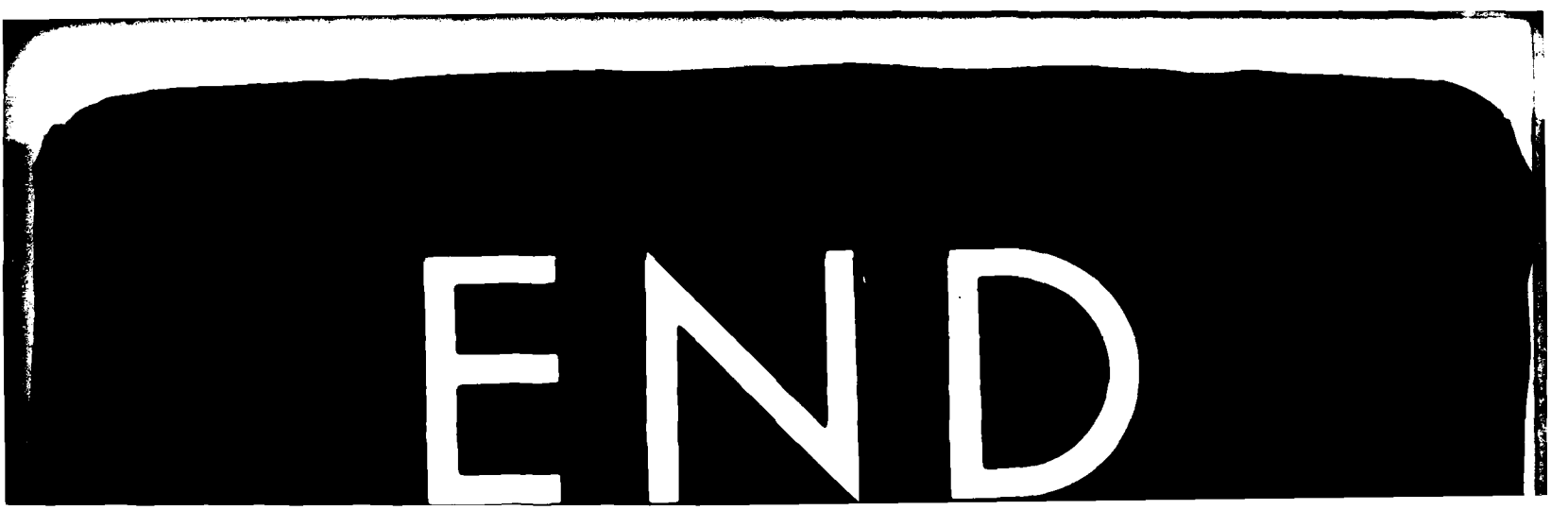
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